DETECTION OF SALMONELLA C_1, D AND V_1 ANTIGENS, BY COAGGLUTINATION, IN BLOOD CULTURES FROM PATIENTS WITH SALMONELLA INFECTIONS.

R.C. ROCKHILL, M. LESMANA, M. A. MOECHTAR AND A. SUTOMO

REPORT NO. TR-868

UNITED STATES NAVAL
MEDICAL RESEARCH UNIT NO. 2
APO SAN FRANCISCO, CALIFORNIA 96528

NAVAL MEDICAL RESEARCH AND DEVELOPMENT COMMAND
BETHESDA, MARYLAND

ADMINISTRATIVE INFORMATION

This study was supported by the Indonesian Ministry of Health and the Naval Medical Research and Development Command, Navy Department for Work Unit MR041.09.002-5037.

Distribution of this document is unlimited

W.H. SCHROEDER
CAPT MSC USN

DETECTION OF *SALMONELLA* C_1, D AND V_1 ANTIGENS, BY COAGGLUTINATION, IN BLOOD CULTURES FROM PATIENTS WITH *SALMONELLA* INFECTIONS

ROBERT C. ROCKHILL, MURAD LESMANA, M. ASRIL MOECHTAR* and ARINI SUTOMO*

U.S. Naval Medical Research Unit No. 2, Jakarta Detachment, Jakarta, Indonesia.
*Rumah Sakit Karantian, Jakarta, Indonesia.

INTRODUCTION

Typhoid fever remains a major disease in developing countries and of an estimated one million infections of *Salmonella typhi* occurring in Africa, Asia and Latin America, 50% of those infected develop the disease and 5% died as a result of complications (Walsh and Warren, 1979). In other studies, the mortality rate among untreated-uncomplicated, treated-complicated and treated-uncomplicated typhoid fever patients was shown to be approximately 15%, 25% and < 5%, respectively (Nourmad and Ziai, 1969; Anderson *et al.*, 1976; Rubin and Weinstein, 1977 and Walsh and Warren, 1979).

Chloramphenicol is the antibiotic most commonly used to treat this infection and is usually given empirically until the culture and antimicrobial sensitivity pattern results are available to indicate otherwise. Other *Salmonella* infections may mimic typhoid fever while the etiologic agent is resistant to chloramphenicol (Rubin and Weinstein 1977). This is especially true in Indonesian where *Salmonella oranienburg* produces an infection with symptoms and a mortality rate nearly identical to typhoid fever. It is isolated as the etiologic agent in approximately 35% of all *Salmonella* enteric fever infections versus 55% for *S. typhi* and is resistant to chloramphenicol in 93% of the instances (unpublished data). Because of the high mortality rate from both of these infections, it is not only important to diagnose typhoid fever early but also exclude it so that appropriate antimicrobial therapy can be continued or changed.

The confirmed diagnosis of typhoid fever and other *Salmonella* infections still rests on the isolation of the etiologic agent (Rubin and Weinstein, 1977). In this study it is shown that *Salmonella* C_1, D and Vi antigens can be detected by the coagglutination (COAG) test 1-2 days before culture and serological results are available, from patients with *S. oranienburg* and *S. typhi* infections. The COAG test is rapid, easy to perform and less expensive than traditional culture and serological methods used to identify these etiologic agents and can possibly be used as a reliable early presumptive indicator of these *Salmonella* infections.

MATERIALS AND METHODS

Patients and bacteriology: Venous blood was drawn from 239 acutely ill patients hospitalized with a tentative diagnosis of typhoid fever in the Infectious Diseases Hospital (Rumah Sakit Karantina), Jakarta during August-December, 1979. Three ml of blood was added to 10 ml of 10% Oxgall *Salmonella* selective medium (Kaye *et al.*, 1966) and the blood cultures (BC) transported

This study was supported by the Indonesian Ministry of Health and the Naval Medical Research and Development Command, Navy Department for Work Unit MR-041.09.002-5037.

The opinions and assertions contained herein are those of the authors and are not to be construed as official or as reflecting the view of the Navy Department or the Naval Service at large or that of the Indonesian Ministry of Health.

Reprint requests to Publications Office, U.S. Naval Medical Research Unit No. 2, APO San Francisco, 96528 or Sarmiento Building, Ayala Avenue, Makati, Metro Manila, Philippines.

to the NAMRU-2 laboratories and incubated at 37 C. MacConkey (MAC), *Salmonella-Shigella* (SS) and desoxycholate citrate lactose sucrose (DCLS) plated agar media (Oxoid, London) were inoculated with aliquots from the BC daily through a period of 7 days or until colonies resembling *Salmonella* were present. Kligler Iron (KIA), lysine iron, motility indole ornithine and urea agar media (Difco) were then inoculated with growth from the plated media. *Salmonella* serological analysis was made with growth from the KIA slant in the 4 tube screen that showed a *Salmonella*-like profile. All antisera were purchased from a commercial source (Difco, Detroit, Michigan).

Coagglutination reagent antiserum production: Vi negative *S.* typhi #0901 containing somatic 0 group D antigen (Institute Pasteur, Paris) was grown on a 5% sheep blood trypticase soy agar slant for 18-20 hours at 37°C. The growth was emulsified with 10 ml of 0.85% sodium chloride in water, the suspension heated in a boiling water bath 2½ hours and the turbidity adjusted to match a MacFarland #7 standard. A New Zealand white rabbit weighing approximately 2 kg was injected intravenously with 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 ml of the *S. typhi* antigen suspension at 3 day intervals. The rabbit was exsanguinated 1 week after the last injection, the blood allowed to clot and serum containing *Salmonella* group D antibodies separated by centrifugation. The same procedure was followed to produce C_1 and Vi antisera using *S. oranienburg* Thompson K8 (Institute Pasteur, Paris) for C_1 antigen and a Vi- producing *Citrobacter freundii* #7851 (Institute Pasteur, Paris) strain.

The antisera were preserved with merthiolate to a final concentration of 0.01% and the antibody titer determined by slide agglutination by the method of Bennett (1977). The reciprocal titer of the group C_1 antibody was $\geq$ 2048, D antibody 1024 and Vi antibody $\geq$ 2048. These antisera were used undiluted to prepare the coagglutination reagents.

Preparation of the stabilized protein A-rich *Staphylococcus:* A Cowan I strain protein A-containing *Staphylococcus aureus* was grown confluently on Mueller-Hinton agar at 37°C for 18 hours. The method of Edwards and Larson (1974) was used to prepare stabilized cells. Basically, the confluent growth was emulsified with 5 ml phosphate buffered saline (PBS: 0.03M potassium phosphate, 0.15M sodium chloride, pH 7.2) and washed X3 with PBS by centrifugation. The *S. aureus* pellet was suspended in 10 ml 0.5% formaldehyde-PBS for 3 hr, washed X3 with PBS and prepared as a 10% suspension in PBS. This suspension was then heated at 80°C for 1 hr with constant stirring, washed X3 with PBS and stored as a 10% stabilized suspension in PBS at 5-10°C.

Staphylococcus-antibody couple: One-tenth ml of each C_1, D and Vi antiserum was mixed with 1 ml of 10% stabilized *S. aureus* suspension and incubated at room temperature for 3 hr with occasional gentle agitation (Kronvall, 1973). Each couple and only stabilized *Staphylococcus*, used as the negative control, was diluted with 9 ml of PBS and labelled C_1-COAG, D-COAG, Vi-COAG and Neg-COAG reagents.

Coagglutination procedure: One tenth ml of BC was mixed with 0.9 ml of 0.12M sodium chloride in a tube. The mixture was heated in a 70 C water bath for 30 minutes to reduce non-specific agglutinins (Jensen, 1958). Fifty microliters of the heated suspension was placed onto 4 areas of a ringed glass slide followed by 50 µl of C_1-COAG, Vi-COAG and neg-COAG reagents and mixed with the BC aliquots. The slide was rotated by hand for 1 minute and the agglutination recorded as 0 (no visible agglutination), 1+ (barely visible), 2+ (moderate), 3+ (heavy) and 4+ (clumps). Agglutination was observed against

an indirect lighted black background and 1–4+ reactions considered positive if the negative control had no visible agglutination.

COAG testing was done at the same time MAC, SS and DCLS media were inoculated with the aliquots from the BC.

RESULTS

Table 1, shows the cumulative percentage positive culture and COAG results from 79 *S. typhi*, 7 *S. paratyphi* A and B and 8 *S. oranienburg* positive BC. The D-andVi-COAG reactions were both positive, 3–4+ with 79 BC that were also positive by isolation of only *S. typhi*. Additionally, 6 *S. paratyphi* A and 1 S. *paratyphi* B isolates from different BC gave 1+ agglutination with the D-COAG reagent. *S. oranienburg* was isolated from 8 BC and the contents of each gave a 3-4+ agglutination with the C_1-COAG reagent. All 3-4+ reactions occurred within 10-15 seconds whereas the 1+ reactions between *S. paratyphi* A and B and the D-COAG reagent became visible only after 45 seconds to 1 minute.

DISCUSSION

The COAG test was found to be a reliable method to detect *Salmonella* C_1, D and Vi antigens in BC from patients with *S. typhi* and *S. oranienburg* bacteremia. The BC had to be incubated at least 18 hours before the antigens could be detected with any degree of certainty. Trials using incubation periods of 2, 4, 6, 8 and 12 hr gave results that were inconsistent. An occasional positive culture, incubated 8 or 12 hr, gave a weak agglutination reaction only for Vi antigen. By 18 hours of incubation sufficient bacterial growth had occurred to provide readily detectable levels of the 3 antigens by the COAG test.

Agglutination was highly dependent on prior treatment of BC aliquots. A preliminary study with 111 *S. typhi* positive and 87 negative BC was done to establish the optimum requirements necessary to assure a sensitive and specific COAG reaction. False positive (30%) agglutination reactions occurred when the aliquots from the 88 negative BC were tested directly and false negative (20%) reactions occurred with the 111 positive BC. When BC aliquots were absorbed for 1 hour with varying volumes of 1% stabilized staphylococci, the false positive reactions were reduced but false negative reactions were similar to the unabsorbed aliquots. Likewise, inconsistent results were obtained when both absorbed and unabsorbed aliquots were heated at 70°C for 30 minutes. The false positive and negative reactions could be

Table 1

Culture and COAG results from *Salmonella* positive blood cultures.

Isolate	Incubation period (days)							Total
	1	2	3	4	5	6	7	
S. typhi	27(34%)*	19(58%)	18(81%)	11(95%)	1(96%)	3(100%)	0	79
	17(22%)	23(51%)	17(73%)	16(93%)	3(97%)	2(99%)	1(100%)	79
S. paratyphi	3(43%)	2(71%)	2(100%)					7
A or B	2(28%)	3(71%)	2(100%)					7
S. oranienburg	4(50%)	3(88%)	1(100%)					8
	4(50%)	4(100%)	0					8

* Number culture positive (cumulative %) number COAG positive (cumulative %)

eliminated by diluting the BC contents 1 : 10 with 0.15 M sodium chloride and adsorbing 0.2 ml with 0.1 ml of 1% stabilized staphylococci or by heating the 1:10 dilution at 70°C for 30 minutes. No agglutination occurred when the COAG reagents were mixed with only the Oxgall medium.

The cumulative percentages of *S. typhi* isolation and COAG positive tests were similar after the fourth day of incubation. During the first 3 days of incubation 7 more BC were positive by isolation than by COAG. If the time of 1-2 days for biochemical and serological identification of *S. typhi* was considered, 51-73% of the positive BC were identified by COAG during the same time it took to identify those 34% positive on day 1 by bacteriological methods. Likewise, all of the *S. oranienburg* positive BC were presumptively identified within 2 days by COAG whereas all of the culture and serological tests were not completed until 2-5 days later.

The minimal cross-reaction between the D-COAG reagent and *S. paratyphi* A and B antigens did not confuse the interpretation because all *S. typhi* were D and Vi antigen positive when tested according to our protocol. With experience, the slight amount of agglutination with the D-COAG reagent was enough to alert us that another *Salmonella* was growing in the BC and therefore the BC should be subcultured to SS, MAC and DCLS media. We now use *S. paratyphi* A and B COAG reagents and observe homologous 3 – 4 + agglutination reactions in 15-30 seconds when these antigens are present in the BC.

The ease and cost of performing the COAG test are other desirable features. The COAG test was performed in less than 1 hour and allowed us to follow the BC until they were positive for C_1 or Vi antigen then subculture to MAC, SS and DCLS for later isolation and identification. This is important since many clinical laboratories in Southeast Asia currently subculture the BC contents only after 1 and 5 days of incubation. We were able to detect the D and Vi antigens in 75% of the *S. typhi* positive BC between days 2-5 that would not otherwise have been subcultured until day 5 in most instances. Likewise, *Salmonella* C_1 antigen was detected in 100% of the BC day 2 whereas 50% could not be confirmed culturally until 5-7 days later. The COAG reagents consisted of only 0.1 ml of antiserum per 10 ml of COAG reagent and was sufficient for 200 tests. This factor is economically significant when compared to the traditional and more expensive culture methods.

SUMMARY

Protein A-containing *Staphylococcus aureus* was coupled to *Salmonella* C_1, D and Vi monovalent antisera to produce C_1-, D- and Vi-COAG reagents. The reagents were used to detect their homologous *Salmonella* antigens in blood cultures (BC). The D and Vi antigens were detected in 79 of 239 BC from patients with suspected typhoid fever and *Salmonella typhi* was later isolated from the same 79 BC. The C_1 antigen was detected in 8 BC from which only *S. oranienburg* was later isolated. The COAG test was generally positive at the same time the BC became culture positive. However, because of subculture and biochemical identification requirements the COAG test could be interpreted 1-2 days before culture results were available. The COAG test can be used to presumptively identify *Salmonella typhi* and *Salmonella* group C_1 in blood cultures before the culture results are available.

ACKNOWLEDGMENTS

The authors would like to acknowledge the technical assistance of Periska Tjaniadi.

REFERENCES

ANDERSON, K.E., JOSEPH, S.W., NASUTION, R., SUNOTO, BUTLER, T., VAN PEENEN, P.F.D., IRVING, G.S., SAROSO, J.S. and WATTEN, R.H., (1976). Febrile illness resulting in hospital admission: A bacteriological and serological study in Jakarta,Indonesia. *Amer. J. Trop. Med. Hyg., 25* : 116.

BENNETT, C.W., (1977). Agglutination tests for febrile diseases. In: *Clinical Serology*. Charles C. Thomas, Springfield, Illinois. p. 144.

EDWARDS, E.A. and LARSON, G.L., (1974). New method of grouping betahemolytic streptococci directly on sheep blood agar plates by agglutination of specifically sensitized protein A containing staphylococci. *J. Clin. Microbiol., 28* : 972.

GADEHOLT, H. and MADSEN, S.T., (1963). Clinical course, complications and mortality in typhoid fever as compared with paratyphoid B. A survey of 2,647 cases. *Acta. Med. Scand., 174* : 753.

JENSEN, K., (1958). A normally occurring *Staphylococcus* antibody in human serum. *Acta. Path. Microbiol. Scand., 44* : 421.

KAYE, D., PALMIERI, M., EYCKMANS, L., ROCHA, H. and HOOK, E., (1966). Comparison of bile and trypticase soy broth for isolation of *Salmonella* from blood. *Amer. J. Clin. Path., 46* : 408.

KRONVALL, G., (1973). A rapid slide-agglutination method for typing pneumococci by means of specific antibody adsorbed to protein A-containing staphylococci. *J. Med. Microbiol., 6* : 187.

NOURMAND, A. and ZIAI, M., (1969). Typhoid and paratyphoid fever in children: Review of symptoms and therapy in 165 cases. *Clin. Pediat., 8* : 235.

RUBIN, R.H. and WEINSTEIN, L., (1977). Clinical features of Salmonellosis In: *Salmonellosis*. Stratton International Medical Book Corporation, New York, N.Y.p. 43.

WALSH, J.A. and WARREN, K.S., (1979). Selective primary health care. An interim strategy for disease control in developing countries. *New Eng J. Med., 301* : 967.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER: NAMRU-2-TR-868	2. GOVT ACCESSION NO.: AD-A103 228	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle): Detection of Salmonella C_1, D and V_1 antigens by coagglutination, in blood cultures from patients with Salmonella infections.		5. TYPE OF REPORT & PERIOD COVERED: Technical Report
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): R.C. Rockhill, M. Lesmana, M. Asril Moechtar, & A. Sutomo.		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS: U.S. Naval Medical Research Unit No. 2, APO San Francisco, Ca. 96528		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: MR-041.09.002-5037
11. CONTROLLING OFFICE NAME AND ADDRESS: Commanding Officer, Naval Medical Research & Development Command, National Naval Medical Center, Bethesda, MD 20014		12. REPORT DATE: 1980
		13. NUMBER OF PAGES: 5
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office): --		15. SECURITY CLASS. (of this report): Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Distribution of this document is unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

--

18. SUPPLEMENTARY NOTES

Published in the Southeast Asian J. Trop. Med. Pub. Hlth., 11(4): 441-445, 1980.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Salmonella infections
Salmonella C_1, D & V_1 antigens
Detection of Salmonella

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Protein A-containing Staphylococcus aureus was coupled to Salmonella C_1, D and Vi monovalent antisera to produce C_1-, D- and Vi-COAG reagents. The reagents were used to detect their homologous Salmonella antigens in blood cultures (BC). The D and Vi antigens were detected in 79 of 239 BC from patients with suspected typhoid fever and Salmonella typhi was later isolated from the same 79 BC. The C_1 antigen was detected in 8 BC from which only S. oranienburg was later

DD FORM 1 JAN 73 1473

UNCLASSIFIED

...over...

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

isolated. The COAG test was generally positive at the same time the BC became culture positive. However because of subculture and biochemical identification requirements the COAG test could be interpreted 1-2 days before culture results were available. The COAG test can be used to presumptively identify Salmonella typhi and Salmonella group C_1 in blood cultures before the culture results are available.